STRATEGIC DIAGNOSTICS INC.

EnviroGard® Endosulfan Plate Kit

75900

Intended Use

The EnviroGard Endosulfan Plate Kit is a quantitative laboratory test for the detection of endosulfan residues in water at 0.075, 0.25, and 1.0 parts per billion (ppb).

Test Principles

The EnviroGard Endosulfan Plate Kit is based on the use of polyclonal antibodies which bind both endosulfan and a endosulfan-enzyme conjugate. The endosulfan in the sample competes with endosulfan-enzyme conjugate for a limited number of antibody binding sites. Antibodies which bind endosulfan are immobilized to the inside of the test wells.

Since there are the same number of antibody binding sites available in every well and each well receives the same number of endosulfan-enzyme conjugate molecules, a sample which contains a low concentration of endosulfan allows the antibody to bind many endosulfan-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of endosulfan allows fewer endosulfan-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to endosulfan concentration.

Darker color = Lower concentration
Lighter color = Higher concentration

Performance Characteristics

The EnviroGard Endosulfan Plate Kit test will not differentiate between endosulfan and certain structurally related compounds but will detect their presence to differing degrees. The following chart shows the value for 50% B_O * and the approximate value for 80% B_O [the Lower Limit of Detection (LLD)] of some related compounds. All concentrations are in ppb.

Compound	LLD	50% B_o
Endosulfan (mixed isomers)	0.08	0.39
Endosulfan (alpha)	0.07	0.32
Endosulfan (beta)	0.08	0.41
Endosulfan Alcohol	0.06	0.35
Endosulfan Sulfate	0.08	0.60
Aldrin	0.32	1.69
Chlordane	0.38	1.80
Dieldrin	0.10	0.60
Endrin	0.06	0.26
Heptachlor	0.69	1.40
Lindane	2.20	19.2
Methoxychlor	>1000	
Toxaphene	3.75	28.5

* $\%B_O$ equals the average optical density (OD) of the calibrator or sample divided by the average OD of the negative control multiplied by 100 (see "Calculate the Results").

Precautions

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not freeze plate kit components or expose them to temperatures greater than 37°C (99°F).
- Allow all reagents and samples to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test.
- Do not store plate kit components for more than 8 hours at ambient temperature.
- Do not expose substrate to direct sunlight.
- Do not use kit components after the expiration date.
- Tightly recap the 1 parts per million (ppm) Endosulfan Calibrator Stock Solution immediately after use to avoid evaporative losses.
- Do not mix reagents or test well strips from plate kits with different lot numbers.
- Use approved methodologies to confirm any positive results.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure; this may give inaccurate results.
- Some solutes and particulates found in untreated ground or surface waters may affect the sensitivity level of this kit.

Materials Provided

The EnviroGard Endosulfan Plate Kit contains the following items:

8 Antibody-Coated Strips (12 wells each), in strip holder
1 vial of 1 ppm Endosulfan (mixed isomers) Calibrator Stock Solution in methanol
1 vial of Endosulfan-Enzyme Conjugate Stock
3 PBS Tablets
1 vial of Substrate
1 vial of Stop Solution

Materials You Provide

You will also need these items:

- disposable-tip, adjustable air-displacement pipette, which will measure 50, 100 and 150 microliters (µl)
- disposable-tip, positive-displacement pipette, which will measure 10 µl
- pipette(s) which will measure 0.3, 0.7, 1.0, 3 and 10 milliliters (ml)
- 10 ml volumetric flask
- graduated cylinder which will measure 200 ml
- deionized water
- borosilicate glass test tubes for working conjugate and calibrator preparation
- marking pen (indelible)
- tape or Parafilm®
- watch or timer (1 hour and 30 minutes)
- clean running tap water or a wash bottle containing tap or deionized water (500 ml) for rinsing wells
- orbital shaker (optional)
- microtiter plate reader or strip reader
- microtiter plate washing device (optional)
- calculator which performs linear regression (optional)
- multi-channel pipette (optional)

Prepare the Working Conjugate

NOTE: The working conjugate should always be prepared fresh just prior to use.

1. Measure 200 mL of deionized water in a graduated cylinder.

2. Add one PBS tablet. Allow to dissolve and mix thoroughly.

3. Add 10 µL of the Conjugate Stock per 1.0 mL of the prepared PBS solution for each 12 well strip that is to be used. Mix thoroughly.

Prepare the Calibrators

The EnviroGard Endosulfan Plate Kit contains a 1 ppm Stock Solution of Endosulfan in methanol. ***Do not use the stock solution directly in the assay.*** This Stock Solution **must** be diluted in laboratory grade water in order to prepare the 0.075, 0.25 and 1.0 ppb calibrators.

NOTE: Accurate pipetting of the Stock Solution and thorough mixing of the calibrator solutions are critical to the performance of the assay.

1. Be certain the 1 ppm Endosulfan Stock Solution is at room temperature. Gently swirl the vial to mix before pipetting.

2. Using a positive-displacement pipette, pipet 10 µL of the 1 ppm Endosulfan Calibrator Stock Solution into a 10 mL volumetric flask. Bring it to volume with deionized water and mix well. This is the 1.0 ppb calibrator.

3. Prepare a 0.25 ppb calibrator by adding 1.0 mL of the 1.0 ppb calibrator to 3.0 mL deionized water, and mix thoroughly.

4. Prepare a 0.075 ppb calibrator by adding 0.3 mL of the 0.25 ppb calibrator to 0.7 mL deionized water, and mix thoroughly.

5. Use deionized water as the negative control in the assay.

NOTE: These aqueous calibrators should be prepared fresh in borosilicate glass test tubes just prior to use.

Perform the Test

The raised markings on the strip holder identify the well location while you add the reagents and samples.

1. Two strips may be used to run the Negative Control, three Calibrators and eight Samples in duplicate. For example:

   Negative Control (C) = deionized water
   Calibrator 1 (C1) = 0.075 ppb
   Calibrator 2 (C2) = 0.25 ppb
   Calibrator 3 (C3) = 1.0 ppb
   Samples (S1, S2, S3, etc.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	C1	C1	C2	C2	C3	C3	S1	S1	S2	S2
B	S3	S3	S4	S4	S5	S5	S6	S6	S7	S7	S8	S8
C												
D												
E												
F												
G												
H												

NOTE: When you use fewer than eight strips, remove the unneeded strips and store them at 4°C to 8°C (39°F to 46°F) in the re-sealable plastic bag (with desiccant) provided.

2. Add **150** µL of Negative Control (C), each Calibrator (C1-C3), and **150** µL of each Sample (S1 to S8) to their respective wells, as shown above.

3. Using the same order of addition, add **50** µL of the **working** Endosulfan-Enzyme Conjugate to each well. Discard any remaining **working** Endosulfan-Enzyme Conjugate.

NOTE: If you are running more than three strips, it is recommended that a multi-channel pipette be used in steps 2, 3, 7 and 9.

4. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for about 1 minute. **B e careful not to spill the contents!**

5. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 1 hour. Orbital mixing at 200 rpm during incubation is preferable, but not mandatory.

6. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with cool running tap water, then shake to empty. Repeat this wash step five times. Invert the plate and tap out as much water as possible. Alternatively, use a microtiter plate washer for the wash steps.

7. Add **100** µL of Substrate to each well, beginning with the Negative Control (C) and Calibrators (C1-C3), and ending with the Samples (S1-S8).

8. Cover the wells with **new** tape or Parafilm and incubate at ambient temperature for 30 minutes. Orbital mixing at 200 rpm is preferable, but not mandatory.

WARNING: Stop Solution is 1N hydrochloric acid. Handle carefully.

9. Add **100** µL of Stop Solution to each well and mix thoroughly. This turns the solution yellow.

NOTE: You should read the plate within 30 minutes of adding the Stop Solution.

Interpret The Results

Spectrophotometric Measurement and Analysis

1. Adjust the wavelength of your microtiter plate reader to 450 nanometers (nm). If your plate reader has dual wavelength capability, use 600 nm or 650 nm as the reference wavelength.

2. If the plate reader does not auto-zero on air, zero the instrument against 200 µL water in a blank well, then measure and record the optical density (OD) of each well. Or, measure and record the OD in every well, then subtract the OD of the water blank from each of the readings.

3. If the microtiter plate reader you are using has data reduction capabilities, use a **semi-log** curve fit for the standard curve. You can also calculate the results manually as described in the next section.

Calculate the Results

1. After the wells have been read, average the OD of each set of calibrators and samples, and calculate the $\%B_O$ as follows:

$$\%B_O = \frac{\text{average OD of calibrator or sample}}{\text{average OD of negative control}} \times 100$$

The $\%B_O$ calculation is used as a means of equalizing different runs of an assay. While the raw OD readings of negative controls, calibrators, and samples are likely to differ from run to run, the $\%B_O$ relationship of calibrators and samples to the negative control should remain fairly constant.

NOTE: The %CV [coefficient of variation = (standard deviation/mean) x 100] for each set of calibrator OD values should not exceed 15%.

2. Graph the $\%B_O$ of each calibrator against its endosulfan concentration on a semi-log scale (see "*Sample Calculations*").

3. Determine the endosulfan concentration of each sample by finding its $\%B_O$ value and the corresponding concentration level on the graph.

4. Interpolation of sample concentration is only valid if the $\%B_O$ of the sample falls within the range of the $\%B_O$'s set by the calibrators. If the $\%B_O$ of a sample is lower than that of the highest calibrator, dilute that sample with laboratory grade water so it falls on the standard curve when you repeat the assay.

Ordering Information

Description	Catalog Number
EnviroGard Endosulfan Plate Kit	75900

Technical Assistance

Strategic Diagnostics Inc.
111 Pencader Drive
Newark, Delaware 19702-3322 USA
800-544-8881
302-456-6789 Phone
302-456-6782 Fax
www.sdix.com
techservice@sdix.com

General Limited Warranty

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